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PROTEKT 1999—2000: a multicentre study of the antimicrobial susceptibility of respiratory tract pathogens in Japan

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Summary

Design: A six-centre study in Japan during the winter of 1999—2000 assessed the in vitro activity of >20 antimicrobial agents against the common respiratory pathogens *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. The minimum inhibitory concentrations (MIC) of each antimicrobial was determined against these isolates using National Committee for Clinical Laboratory Standards (NCCLS) methodology.
Results: Among *S. pneumoniae* isolates, 44.5% were penicillin resistant. The macrolide resistance rate was 77.9% with 90.5% of penicillin-resistant strains also being macrolide resistant. Resistance mechanisms in macrolide-resistant isolates were identified as *mef*(A) or *erm*(B) in 42.5% and 52.5%, respectively. Of the fluoroquinolone-resistant isolates (1.3%), most were also penicillin and macrolide resistant. All strains were inhibited by telithromycin at ≤1 mg/L. Among *S. pyogenes* isolates, erythromycin resistance was 17.5% overall but showed considerable variation among the six centres. For *H. influenzae*, 8.5% produced β-lactamase and a single β-lactamase-negative, ampicillin-resistant isolate (0.36%) was obtained, and there was no fluoroquinolone resistance. All isolates were susceptible to telithromycin.

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Most antimicrobials showed good activity against *M. catarrhalis*, although 96.7% were β-lactamase positive.
Conclusion: The prevalence of antimicrobial resistance to macrolides, penicillin and the fluoroquinolones among the common respiratory pathogens is high in Japan.

Introduction

The prevalence of resistant isolates of common bacterial respiratory tract pathogens is increasing, and nowhere more so than in Asia. In some Asian countries, penicillin resistance may be as high as 70%.[1–3] In the last decade, macrolide resistance has also increased dramatically, exceeding penicillin resistance in some areas,[2] and growing resistance to chloramphenicol, co-trimoxazole and tetracycline continues relentlessly.[4]

Most respiratory tract infections are viral in origin but are frequently followed by secondary infections resulting from opportunistic invasion by commensal respiratory bacteria. The four most important bacterial pathogens associated with community-acquired upper and lower respiratory tract infections (RTIs — acute/chronic sinusitis, acute/chronic otitis media, acute/chronic pharyngitis, community-acquired pneumonia, acute bacterial exacerbation of chronic bronchitis and acute bacterial exacerbation of chronic obstructive airways disease) are *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. Less commonly, atypical and intracellular pathogens including *Legionella pneumophila*, *Mycoplasma pneumoniae*, and *Chlamydophila (Chlamydia) pneumoniae* are also found as causes of community-acquired RTIs.[5–7]

S. pneumoniae in particular has acquired resistance to several classes of antimicrobial compounds, including penicillins, macrolides and fluoroquinolones, by a variety of mechanisms.[8] For *Haemophilus* species and *M. catarrhalis*, β-lactamase production is the principal mechanism of resistance to penicillins and cephalosporins. The choice of antimicrobial therapy in community-acquired RTIs is generally empirical and complicated by increasing bacterial resistance. Effective strategies for ensuring adequate antimicrobial therapy are therefore necessary but may only be achieved through an understanding of the geographic variation in resistance and by monitoring trends in resistance development.

Established in 1999, PROTEKT (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin) is an international surveillance study to chart the prevalence of important resistance phenotypes and examine the susceptibility of community-acquired RTI pathogens to a range of antimicrobial compounds. Telithromycin is the first ketolide antibacterial to be approved for clinical use for the treatment of upper and lower RTIs. With over 35 countries and 500 centres now participating, PROTEKT is able to concentrate on defining trends in specific regions and countries. Detailed data from the examination of isolates of *S. pneumoniae*, *H. influenzae*, *M. catarrhalis* and *S. pyogenes* collected during the 1999—2000 winter season in Japan are now presented and, where possible, related to trends seen in previous studies.[2,9,10]

Materials and methods

Participating centres

During the 1999—2000 winter season, six centres took part in the study: Kanagawa, Sendai, Tokyo (two centres), Nagasaki and Osaka.

Bacterial isolates

Centres were asked to collect the following isolates from patients with community-acquired upper and lower RTIs: ≥40 isolates each of *S. pneumoniae* and *H. influenzae*, ≥25 of *S. pyogenes*, and ≥20 of *M. catarrhalis*. Sources for isolates were cultures from blood, sputum, bronchoalveolar lavage, middle ear fluid, nasopharyngeal swab or aspirate, and sinus aspirate. Duplicate strains or strains originating from previous collections were not accepted.

Identification and antimicrobial susceptibility testing

Isolates were identified at source and re-identified at the central laboratory by methods previously described in detail.[11] Minimum inhibitory concentrations (MICs) were determined using previously described broth microdilution methods,[11] according to the National Committee for Clinical Laboratory Standards (NCCLS) of the USA guidelines, for the following antimicrobial agents: amoxicillin—clavulanate, cefaclor, cefcapene, cefdinir, cefditoren,

cefixime, cefpodoxime, cefuroxime, telithromycin, erythromycin, roxithromycin, clarithromycin, azithromycin, rokitamycin, minocycline, tetracycline, ciprofloxacin, levofloxacin, sparfloxacin and tosufloxacin. MICs were also determined for penicillin and clindamycin against *S. pneumoniae* and *S. pyogenes* isolates and for ampicillin and amoxicillin against *H. influenzae* and *M. catarrhalis* isolates. Test results were acceptable only if the MICs for the control strains were within performance range. The following control strains were used: *S. aureus* ATCC 29213, *E. coli* ATCC 25922 and ATCC 35218, *H. influenzae* ATCC 49766, *H. influenzae* ATCC 49247, and *S. pneumoniae* ATCC 49619.

Breakpoint concentrations used to interpret MIC data qualitatively were based upon those published by the NCCLS of the USA,[12] where available. For telithromycin, NCCLS approved (SAST 2003) breakpoints were applied: *S. pneumoniae*: susceptible $\leq$1 mg/L, intermediate 2 mg/L, resistant $\geq$4 mg/L; and for *H. influenzae*: susceptible $\leq$4 mg/L, intermediate 8 mg/L, resistant $\geq$16 mg/L. No NCCLS breakpoints are available for *S. pyogenes* or *M. catarrhalis*.

β-lactamase detection

β-lactamase activity was detected using the chromogenic cephalosporin (nitrocefin) test (Unipath Ltd. Basingstoke, UK).

Macrolide resistance mechanism detection

For *S. pneumoniae*, the presence of resistance mechanisms for both MLS_B (*erm*) and M-resistance (*mef*) was analysed using a rapid-cycle multiplex PCR method with probe detection. This method detects *erm*(A), *erm*(A) subclass *erm*(TR), *erm*(B), *erm*(C), and *mef*(A) genes.[13]

Results

Streptococcus pneumoniae

A total of 308 *S. pneumoniae* isolates from the six participating centres were tested. The prevalence of penicillin resistance (MIC $\geq$2 mg/L) was 44.5% overall and ranged narrowly between 44.2% and 48.4% for five of the six centres, with Osaka lower at 36.4%. Penicillin-intermediate (MIC 0.12—1 mg/L) isolates (19.8% overall) were less evenly distributed, with centres reporting between 7.7% (Kanagawa) and 31.6% (Sendai) (Table 1). The prevalence of macrolide resistance (erythromycin MIC $\geq$1 mg/L) was 77.9%, far exceeding that of penicillin resistance, and ranged from 67.3% (Kanagawa) to 86.4% (Osaka) (Table 1). Only one strain was of the intermediate type (erythromycin MIC 0.5 mg/L). Almost half of all *S. pneumoniae* isolates (40.3%) were co-resistant to penicillin and erythromycin (macrolide) (Table 1).

Of the 239 macrolide-resistant isolates of *S. pneumoniae* analysed for their resistance mechanism, 52.7% carried *erm*(B) (MLS_B resistance) and 42.7% carried *mef*(A) (efflux resistance), with 3.3% (n = 8) of isolates carrying both mechanisms (*mef*(A)+*erm*(B)) (Table 2). *erm*B isolates were evenly distributed across the three penicillin resistance phenotypes, whereas *mef*(A) resistance was associated predominantly with penicillin-resistant (70.6%) rather than penicillin-susceptible (16.7%) isolates.

Among the β-lactams, the most active were cefditoren (MIC_{90} 1 mg/L, 98.4% of all isolates susceptible) and amoxicillin—clavulanate (MIC_{90} 2 mg/L, 96.4% of all isolates susceptible). Both retained >90% activity among the penicillin- and macro-

Table 1 Penicillin and macrolide susceptibility and cross-resistance of *Streptococcus pneumoniae* isolates from Japan.

Centre	No. of isolates	Pen-I[a]		Pen-R[b]		Mac-R[c]		Pen-R/Mac-R	
		n	%	*n*	%	*n*	%	*n*	%
Kanagawa	52	4	7.7	23	44.2	35	67.3	18	34.6
Sendai	38	12	31.6	18	47.4	32	84.2	18	47.4
Tokyo 1	54	11	20.4	24	44.4	43	79.6	22	40.7
Tokyo 2	62	10	16.1	30	48.4	48	77.4	27	43.5
Nagasaki	58	14	24.1	26	44.8	44	75.9	23	39.7
Osaka	44	10	22.7	16	36.4	38	86.4	16	36.4
Total	308	61	19.8	137	44.5	240	77.9	124	40.3

[a] Penicillin-intermediate: MIC 0.12—1 mg/L.
[b] Penicillin-resistant: MIC $\geq$ 2 mg/L.
[c] Erythromycin-resistant: MIC $\geq$ 1 mg/L.

Table 2 Effect of specific macrolide-resistance mutations for 239 macrolide-resistant isolates of *Streptococcus pneumoniae* from Japan and classified by penicillin susceptibility phenotype.

Genotype	MAC-R[a]		MIC range (mg/L)	PEN-S[b]		PEN-I[c]		PEN-R[d]	
	n	%		*n*	%	*n*	%	*n*	%
mef(a)	102	42.7	1—≥128	17	16.7	13	12.7	72	70.6
erm(b)	126	52.7	32—≥128	44	34.9	34	27.0	48	38.1
mef(a) + *erm*(b)	8	3.3	64—≥128	2	25	2	25	4	50
None specified	3	1.3	64—≥128	3	100	0	0	0	0

[a] Macrolide-resistant (erythromycin MIC ≥1 mg/L).
[b] Penicillin-susceptible: MIC ≤ 0.06 mg/L.
[c] Penicillin-intermediate: MIC 0.12—1 mg/L.

lide-resistant isolates (Table 3). With the exception of telithromycin and the fluoroquinolones, susceptibility to non-β-lactams was low (Table 3). Among the penicillin-resistant isolates, <10% were susceptible to macrolides and tetracycline.

Erythromycin, roxithromycin, azithromycin and clarithromycin gave typical trimodal MIC distributions with clusters of isolates inhibited by 0.06—0.12 mg/L, 2—4 mg/L and >32—>64 mg/L (Figure 1). Small numbers of isolates were inhibited

Table 3 Comparative in vitro activity and percentage susceptibility of various antimicrobials against penicillin-intermediate, penicillin-resistant and erythromycin-resistant isolates of *Streptococcus pneumoniae* from Japan using NCCLS (2002) interpretative breakpoints.

Antimicrobial	All isolates (*n* = 308)			PEN-I[a] (*n* = 61)			PEN-R[b] (*n* = 137)			MAC-R[c] (*n* = 240)		
	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[d]	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[d]	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[d]	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[d]
Penicillin	0.5	4	35.7	0.25	1	0	2	4	0	2	4	27.9
Amoxicillin—clavulanate[e]	0.5	2	96.4	0.25	0.5	100	2	2	92.0	0.5	2	95.8
Cefaclor	16	>64	20.8	16	64	8.2	64	>64	0	2	4	11.3
Cefcapene	2	4	NA	2	4	NA	4	4	NA	2	4	NA
Cefdinir	4	8	44.5	2	4	41.0	8	8	2.2	4	8	35.8
Cefditoren	0.5	1	98.4	0.5	1	96.7	1	1	97.8	0.5	1	97.9
Cefixime	16	32	—[f]	16	64	—[f]	32	64	—[f]	32	64	—[f]
Cefpodoxime	2	4	40.3	2	4	36.1	2	4	0	2	4	32.1
Cefuroxime	4	8	41.2	4	8	37.7	8	8	0	4	8	33.3
Telithromycin	0.06	0.25	100[g]	0.06	0.5	100[g]	0.06	0.12	100[g]	0.06	0.25	100[g]
Erythromycin	8	>64	21.8	64	>64	19.7	4	>64	9.5	64	>64	0
Roxithromycin	8	>32	NA	>32	>32	NA	8	>32	NA	64	64	NA
Clarithromycin	4	>32	22.1	32	>32	19.7	4	>32	9.5	32	>32	0
Azithromycin	8	>64	21.8	64	>64	18.0	8	>64	9.5	64	>64	0
Rokitamycin	0.12	>32	NA	1	>32	NA	0.12	>32	NA	1	64	NA
Clindamycin	0.12	>4	54.9	4	>4	41.0	0.12	>4	62.0	4	>4	42.1
Minocycline	8	16	NA	16	16	NA	8	16	NA	16	16	NA
Tetracycline	>16	>16	20.8	16	>16	18.0	>16	>16	9.5	>16	>16	4.2
Ciprofloxacin	1	2	NA	1	2	NA	1	2	NA	1	2	NA
Levofloxacin	1	1	96.4	0.5	1	100	1	1	97.1	1	1	96.3
Sparfloxacin	0.25	0.5	96.1	0.25	0.25	100	0.25	0.25	96.4	0.25	0.5	95.8
Tosufloxacin	0.12	0.12	NA	0.06	0.12	NA	0.06	0.12	NA	0.12	0.12	NA

[a] Penicillin-intermediate: MIC 0.12—1 mg/L.
[b] Penicillin-resistant: MIC ≥2 mg/L.
[c] Erythromycin-resistant: MIC ≥1 mg/L.
[d] % of isolates susceptible.
[e] Also applies to amoxicillin.
[f] Susceptibility predicted from penicillin.
[g] NCCLS (SAST Jan 2003) approved breakpoint for telithromycin: susceptible ≤1 mg/L; NA = NCCLS breakpoints not available.

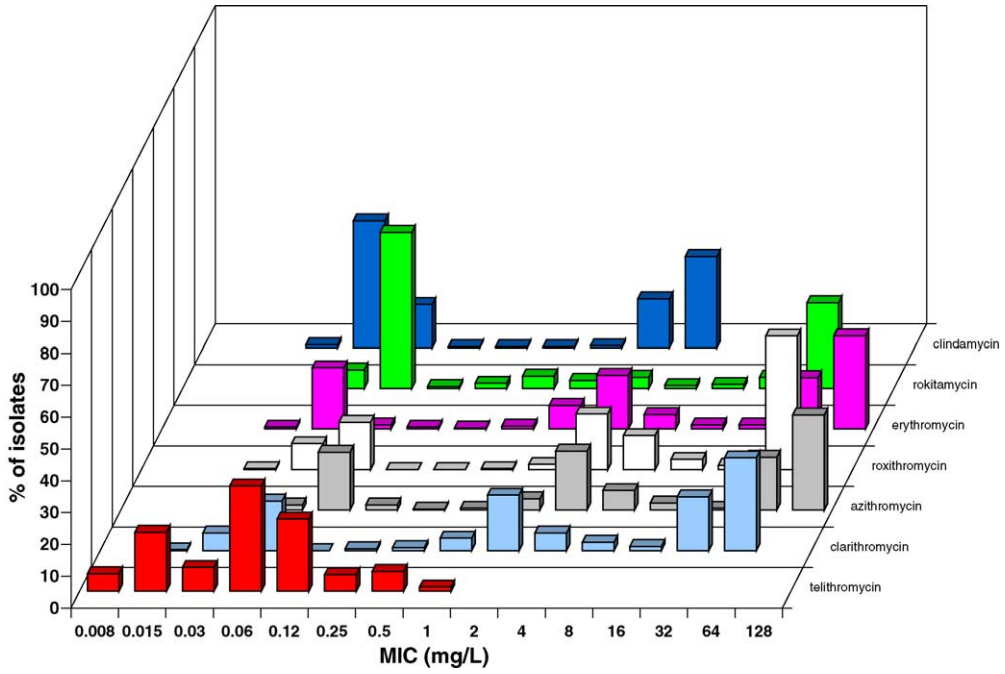

Figure 1 MIC distribution for macrolide-lincosamide-streptogramin (MLS) class antimicrobials against *Streptococcus pneumoniae* from Japan.

by each inter-mode concentration of each antimicrobial. Rokitamycin and clindamycin showed two obvious clusters in their MIC distributions, with just under half the isolates inhibited within the lowest concentration cluster. This was reflected in the MIC_{50} (0.12 mg/L) for rokitamycin and clindamycin, which differed considerably from the four macrolides with typical trimodal MIC distributions (MIC_{50} 4—8 mg/L).

Telithromycin showed much lower mode MIC (0.06 mg/L) and MIC_{90} (0.25 mg/L) than the macrolides (Figure 1). Among the macrolide-resistant isolates, the telithromycin MIC_{90} value was markedly higher for the *erm*(B) genotype (0.5 mg/L) than the *mef*(A) genotype (0.12 mg/L). Despite a shift upwards in the distribution of telithromycin MIC values among the macrolide-resistant isolates (particularly among the eight *erm*(B)+*mef*(A) strains (Figure 2)) compared with macrolide-susceptible isolates (telithromycin MIC_{90} 0.015 mg/L), all isolates were susceptible to telithromycin at $\leq$1 mg/L.

Fluoroquinolone resistance (levofloxacin MIC $\geq$8 mg/L) was 1.3% overall, with little variation among centres. Of the four fluoroquinolone-resistant isolates, three were penicillin-resistant and one was penicillin-susceptible. All four fluoroquinolone-resistant isolates were also macrolide- and tetracycline-resistant. Susceptibility to telithromycin was unaffected by fluoroquinolone resistance. Overall, of those antibacterial agents tested, the most active against *S. pneumoniae* in the winter season 1999—2000 in Japan (in terms of potency and susceptibility percentage) were telithromycin, sparfloxacin, levofloxacin, cefditoren and amoxicillin—clavulanate.

Streptococcus pyogenes

The most potent antimicrobial against *S. pyogenes* isolates was penicillin (MIC_{90} 0.008 mg/L) against which all 120 isolates were susceptible. Macrolide resistance showed considerable variation among the six centres, with the highest prevalence (42.1%) in Sendai and 0% in Nagasaki (although this centre collected only three isolates). Overall, 82.5% of isolates were erythromycin-susceptible. Among the 21 (17.5%) erythromycin-resistant isolates, the mechanisms of resistance detected were *mef*(A) in 15 isolates, *erm*(A) subclass *erm*(TR) in five isolates and *erm*(B) in one isolate. Telithromycin had mode MIC (0.015 mg/L) and MIC_{90} (0.25 mg/L), values which were 16- to 32-fold lower than those of the tested macrolides.

Haemophilus influenzae

β-lactamase production amongst *H. influenzae* isolates (n = 281) had an overall incidence of 8.5% and variation among centres of 5.1% to 11.5%. A single β-lactamase-negative, ampicillin-resistant (MIC $\geq$4 mg/L) strain (BLNAR) was identified (from Sendai). A further nine β-lactamase-negative isolates,

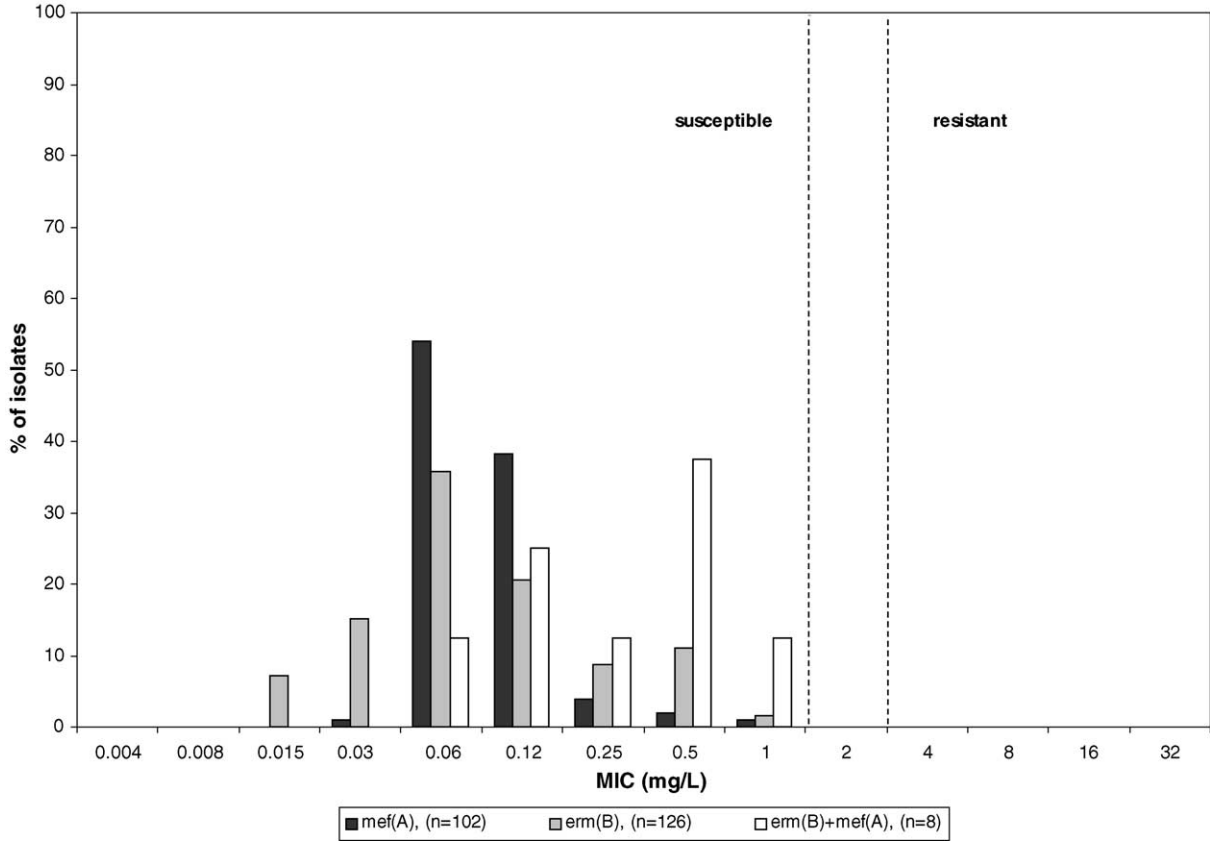

Figure 2 Telithromycin MIC distribution for macrolide-resistant genotypes of *Streptococcus pneumoniae* from Japan.

however, had ampicillin MICs of 2 mg/L (intermediate resistance according to NCCLS breakpoints).

Comparative in vitro activity of all antimicrobial compounds tested against *H. influenzae* and categorised by β-lactamase production is shown in Table 4. Of the β-lactams tested, cefditoren (MIC_{90} 0.06 mg/L; no NCCLS breakpoint) and cefixime (MIC_{90} 0.25 mg/L; 100%) were the most active.

Chloramphenicol resistance had low prevalence (3.6%), with nine of the ten nonsusceptible isolates also β-lactamase-positive. Similarly, tetracycline resistance was low (6.4%) with resistant isolates predominantly β-lactamase-positive (12/18). The MIC_{90} values for both chloramphenicol and tetracycline among β-lactamase-positive *H. influenzae* isolates (16 mg/L) were 16 times greater than for β-lactamase-negative isolates (Table 4).

The MICs of the macrolides and telithromycin to *H. influenzae* isolates followed unimodal distributions in the rank order: azithromycin (MIC_{90} 1 mg/L) $>$ telithromycin (MIC_{90} 2 mg/L) $>$ rokitamycin (MIC_{90} 8 mg/L) $>$ clarithromycin and roxithromycin (MIC_{90} 16 mg/L), (Table 4). There was no correlation between ketolide/macrolide susceptibility and β-lactamase production.

Moraxella catarrhalis

Of the 122 *M. catarrhalis* isolates, 118 (96.7%) were β-lactamase-positive. With the exception of some β-lactams (ampicillin, cefaclor, cefuroxime and cefcapene), all antimicrobials tested showed good activity (MIC_{90} values of $\leq$1 mg/L) against *M. catarrhalis* isolates (Table 4). Cefixime was the most active β-lactam (MIC_{90} 0.25 mg/L), followed by cefdinir and cefditoren (both, MIC_{90} 0.5 mg/L) (Table 4). The rank order of activity of the MLS class of antimicrobials was azithromycin (MIC_{90} 0.06 mg/L) $>$ telithromycin, clarithromycin, and rokitamycin (MIC_{90} 0.25 mg/L) $>$ roxithromycin (MIC_{90} 0.5 mg/L). Sparfloxacin and tosufloxacin were the most potent (MIC_{90} 0.008 mg/L) fluoroquinolones.

Discussion

Streptococcus pneumoniae

Previous reports have demonstrated the increasing prevalence of penicillin resistance of both intermediate (MIC 0.12—1 mg/L) and resistant (MIC

Table 4 Comparative in vitro activity of various antimicrobials against isolates of *Haemophilus influenzae* and *Moraxella catarrhalis* from Japan.

Antimicrobial	*Haemophilus influenzae*									*Moraxella catarrhalis*[a]	
	All isolates (n = 281)			β-lactamase positive (n = 24)			β-lactamase negative (n = 257)			All isolates (n = 122)	
	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[b]	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[b]	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S[b]	MIC_{50} (mg/L)	MIC_{90} (mg/L)
Ampicillin	0.25	2	87.9	>16	>16	0	0.25	1	96.1	8	16
Amoxicillin–clavulanate	0.5	2	99.3	1	2	100	0.5	2	99.2	0.12	0.25
Cefaclor	4	16	86.5	16	32	45.8	4	8	90.3	2	16
Cefcapene	0.5	1	NA	1	16	NA	0.5	4	NA	8	16
Cefdinir	0.25	1	91.8	0.5	2	79.2	0.25	1	93.0	0.12	0.5
Cefditoren	0.015	0.06	NA	0.015	0.12	NA	0.015	0.03	NA	0.12	0.5
Cefixime	0.03	0.25	100	0.12	0.5	100	0.03	0.25	100	0.25	0.25
Cefpodoxime	0.06	0.5	99.3	0.12	1	100	0.06	0.5	99.2	0.5	1
Cefuroxime	1	4	95.4	2	4	95.8	1	4	95.3	2	4
Telithromycin	1	2	100[c]	1	2	100[c]	1	2	100[c]	0.06	0.25
Roxithromycin	8	16	NA	8	8	NA	8	16	NA	0.25	0.5
Clarithromycin	8	16	88.3	8	16	75.0	8	16	89.5	0.25	0.25
Azithromycin	1	1	100	1	2	100	1	1	100	0.06	0.06
Rokitamycin	4	8	NA	4	8	NA	4	8	NA	0.25	0.25
Minocycline	1	2	NA	1	2	NA	1	2	NA	0.06	0.06
Tetracycline	0.5	1	93.6	1	16	50.0	0.5	1	97.7	0.25	0.5
Co-trimoxazole	0.06	0.06	97.9	0.06	4	87.5	0.06	0.06	98.8	0.12	0.25
Chloramphenicol	0.5	1.0	96.4	0.5	8	62.5	0.5	0.5	99.6	0.5	0.5
Ciprofloxacin	0.015	0.015	100	0.015	0.03	100	0.015	0.015	100	0.03	0.03
Levofloxacin	0.015	0.015	100	0.015	0.03	100	0.015	0.015	100	0.03	0.03
Sparfloxacin	0.004	0.008	99.3	0.008	0.008	100	0.004	0.008	99.2	0.008	0.008
Tosufloxacin	0.004	0.008	NA	0.008	0.008	NA	0.004	0.008	NA	0.008	0.008

[a] NCCLS breakpoints not available for *M. catarrhalis*.
[b] % of isolates susceptible according to NCCLS breakpoints.
[c] NCCLS (SAST 2003) approved breakpoint for *H. influenzae*: susceptible ≤4 mg/L; NA = NCCLS breakpoints not available.

≥2 mg/L) phenotypes amongst isolates of *S. pneumoniae*.[2,9,14] During the 1999–2000 winter season, 44.5% of *S. pneumoniae* RTI isolates from Japan were penicillin resistant and 19.8% were penicillin intermediate, a pattern with small geographic variation throughout Japan (Table 1). In previous studies, Yoshida et al.[15] found that penicillin resistance increased from 4.3% in 1988 to 9.8% in 1992 and Sahm et al.[10] reported 10.1% penicillin resistance for the 1997–98 winter season. Therefore, penicillin resistance in Japan is increasing and current data strongly suggest that the trend has accelerated in recent years.

Resistance to penicillin in *S. pneumoniae* is mediated by changes in the affinity of high molecular weight penicillin binding proteins (PBPs) for their substrates. As these PBPs are also targets for other β-lactams, the activity of aminopenicillins, cephalosporins and carbapenems is also reduced against penicillin-resistant strains. This is most evident with compounds considered active only against penicillin-susceptible *S. pneumoniae*, such as cefaclor and cefixime. Cefuroxime, cefpodoxime and cefdinir retained some activity against penicillin-intermediate isolates (approximately 40%), but little or no activity against resistant isolates. This perhaps reflects the trend towards greater resistance as previous work has shown that cefuroxime, among other cephalosporins, can retain activity against many penicillin-resistant strains.[16,17] The most effective β-lactams for the 1999–2000 winter season in Japan were cefditoren and amoxicillin–clavulanate, with over 90% susceptibility among penicillin-resistant strains. The amoxicillin–clavulanate results can be extrapolated to include amoxicillin as an effective β-lactam (92% susceptibility among penicillin-resistant strains), although amoxicillin itself was not tested against *S. pneumoniae*.

Macrolides form the principal alternative to β-lactams for the treatment of lower RTIs involving *S. pneumoniae*. However, it is now clear that this class of compounds, including erythromycin, clarithromycin and azithromycin, is seriously compromised by the development of resistance not only as a result of

the increasing prevalence of penicillin-resistant pneumococci but also, in Japan, among penicillin-susceptible strains.

Typical of the Far East, *S. pneumoniae* macrolide resistance in Japan is high (77.9%) with some centre variation (67.3—86.4%). This finding of 77.9% is considerably higher than the 66.5% reported for the 1997—1998 winter season.[10] The proportion of penicillin-resistant isolates ($n = 137$) that are also macrolide-resistant has not increased over the same period (124/137, 90.5%) and is slightly lower than the previous study (1997—1998, 95.5%).

Two main mechanisms are known to account for macrolide resistance in *S. pneumoniae*. With the first, resistance is associated with specific mutation within the *erm* gene that confers resistance to most macrolides, lincosamides and streptogramin B antibiotics.[18] With the second, the so-called M phenotype, resistance is mediated by an efflux mechanism due to the presence of the *mef*(A) gene that confers resistance to 14- and 15-membered macrolides.[19] Growing macrolide resistance is of increasing concern, especially that dependent upon the *erm*(B) genotype; not only because it is the more potent macrolide resistance, but because resistance to other antimicrobial compounds appear preferentially to be associated with it. This study shows that in Japan, the distribution of *erm*(B) and *mef*(A) are similar.

Telithromycin, a synthetic ketolide derived by chemical modification of desclarithromycin, was designed to maintain potent antimicrobial activity against community-acquired respiratory tract infection (CARTI) pathogens, even macrolide-resistant pneumococci, and not to induce resistance due to *erm*(B).[20] There was, however, an upward shift in telithromycin MICs among the isolates with *erm*(B)-mediated macrolide resistance compared with *mef*(A) strains. This effect of *erm*(B) resistance on the activity of telithromycin has been reported previously although, as in this study, all the isolates were still found to be inhibited by telithromycin at $\leq$1 mg/L.

Worldwide incidence of fluoroquinolone-resistant *S. pneumoniae* (levofloxacin MIC $\geq$8 mg/L) is rare, although it tends to be concentrated in pockets of Asia (specifically Hong Kong) and North America. The four (1.3%) resistant isolates from Japan were obtained from four different centres, and would therefore suggest random distribution and independent origin.

Streptococcus pyogenes

Streptococcus pyogenes was susceptible to most of the antimicrobials tested with the notable exception of the macrolides (17.5% resistant, mostly *mef*(A)). Telithromycin was 16- to 32-fold more potent than the macrolides although penicillin remains the most potent antimicrobial.

Haemophilus influenzae

There is considerable variability worldwide in the prevalence of β-lactamase production by *H. influenzae*, with previous studies showing values of 19% for Europe, 42% for the USA and around 14% for Japan.[10,21,22] The value for Japan is slightly higher than the finding here of 8.5%. Only a single (0.36%) β-lactamase-negative ampicillin-resistant (BLNAR) (ampicillin MIC $\geq$4 mg/L) strain was isolated in Japan during the winter season 1999—2000, although 3.2% of isolates were β-lactamase-negative with low-level resistance to ampicillin (MIC 2 mg/L). These values are considerably lower that those published for Japan by Hasegawa et al.[23]

Of the β-lactams tested, cefixime (100%), cefpodoxime (99.3%), cefuroxime (95.4%), and cefdinir (91.8%) were the most active, followed by ampicillin (87.9%), cefaclor (86.5%), and amoxicillin (81.5%), (Table 4). β-lactamase production conferred resistance to ampicillin and amoxicillin for all isolates, but had little or no effect on susceptibility to cefixime, cefpodoxime, and cefuroxime. For cefdinir and cefaclor the effect was partial, susceptibility being reduced by approximately 15% and 50%, respectively.

Similar partial co-resistance was observed for chloramphenicol and tetracycline, where 99.6% and 97.7% β-lactamase-negative isolates were susceptible compared with 62.5% and 50% β-lactamase-positive isolates, respectively.

All isolates were susceptible to azithromycin, with 88.3% susceptible to clarithromycin. For the 1997—1998 winter season, Sahm et al. also found 100% susceptibility of isolates to azithromycin,[10] with 93.2% susceptible to clarithromycin, indicating a slightly increased resistance towards this macrolide. In 1999—2000, the azithromycin MICs for the Japanese isolates were all $\leq$2 mg/L. All isolates of *H. influenzae* were susceptible to the ketolide telithromycin at $\leq$4 mg/L.

Moraxella catarrhalis

β-lactamase production was observed in 96.7% of *M. catarrhalis* isolates tested in Japan, a figure almost identical to 97.5% reported by Sahm et al.[10] for the 1997—98 winter season. β-lactamase-producing strains of *M. catarrhalis* were first reported in the late 1970s and by the late 1980s, these strains were predominant, accounting for more than 80% of clinical isolates in a number of studies.[8,24,25]

The β-lactamases of *M. catarrhalis* are inhibited by clavulanic acid and the combination of amoxicillin—clavulanic acid has been shown to be highly active against this species.[25—29] Indeed, in this study, among β-lactamase-positive *M. catarrhalis* the MIC_{90} for unprotected ampicillin was high at 16 mg/L, in contrast with 0.25 mg/L for amoxicillin—clavulanate.

Summary

Despite growing public awareness Japan has witnessed increased and even accelerating resistance to the macrolides and to β-lactams. Fluoroquinolone resistance, albeit at a low level, would also appear to be endemic. This study documents the high prevalence of antimicrobial resistance and co-resistance among respiratory pathogens in Japan.

For a great proportion of respiratory infections that require antimicrobial therapy, amoxicillin remains largely effective; however, in Japan, the preference is for the use of newer drugs as first-line treatment. This study reinforces the necessity for judicious use of old and new antimicrobial compounds and, with the technical ability that is now available, to evaluate resistance at a genetic level to monitor more detailed patterns of emergence.

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References

1. Dagan R, Klugman KP, Craig WA, Baquero F. Evidence to support the rationale that bacterial eradication in respiratory tract infection is an important aim of antimicrobial therapy. *J Antimicrob Chemother* 2001;**47**:129—40.
2. Felmingham D, Grüneberg RN. The Alexander Project 1996—1997: latest susceptibility data from this international study of bacterial pathogens from community-acquired lower respiratory tract infections. *J Antimicrob Chemother* 2000;**45**:191—203.
3. Baquero F. Pneumococcal resistance to β-lactam antibiotics: A global geographic overview. *Microb Drug Resist* 1995;**1**: 115—20.
4. Song JH, Lee NY, Ichiyama S. The Asian Network for Surveillance of Resistant Pathogens (ANSORP) Study Group. Spread of drug-resistant *Streptococcus pneumoniae* in Asian countries: Asian Network for Surveillance of Resistant Pathogens (ANSORP) study. *Clin Infect Dis* 1999;**28**:1206—11.
5. Karlowsky JA, Thornsberry C, Critchley IA, et al. Susceptibilities to levofloxacin in *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* clinical isolates from children: results from 2000—2001 and 2001—2002 TRUST studies in the United States. *Antimicrob Agents Chemother* 2003;**47**:1790—7.
6. Hu YY, Yu SJ, Liu G, Gao W, Yang YH. Antimicrobial susceptibility of *Haemophilus influenzae* among children in Beijing, China, 1999—2000. *Acta Paediatr* 2002;**91**:136—40.
7. Grüneberg RN, Felmingham D. Results of the Alexander Project: a continuing, multicenter study of the antimicrobial susceptibility of community-acquired, lower respiratory tract bacterial pathogens. *Diagn Microbiol Infect Dis* 1996;**25**:169—81.
8. Mandell LA. Community-acquired pneumonia: Etiology, epidemiology and treatment. *Chest* 1995;**108**:35S—42S.
9. Ball P. Epidemiology and treatment of chronic bronchitis and its exacerbations. *Chest* 1995;**108**:43S—52S.
10. Goldstein F, Bryskier A, Appelbaum PC, et al. The etiology of respiratory tract infections and the antibacterial activity of fluoroquinolones and other oral bacterial agents against respiratory pathogens. *Clin Microbiol Infect* 1998;**4**:2S8—18.
11. Felmingham D. Antibiotic resistance: Do we need new therapeutic approaches? *Chest* 1995;**108**:70S—8S.
12. Sahm DF, Jones ME, Hickey ML, Diakun DR, Mani SV, Thornsberry C. Resistance surveillance of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* isolated in Asia and Europe, 1997—1998. *J Antimicrob Chemother* 2000;**45**:457—66.
13. Felmingham D. The need for antimicrobial resistance surveillance. *J Antimicrob Chemother* 2002;**50**:1—7.
14. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing — twelfth informational supplement M100—S12. NCCLS, Wayne, PA, USA, 2002.
15. Farrell DJ, Morrissey I, Bakker S, Felmingham D. Detection of macrolide resistance mechanisms in *Streptococcus pneumoniae* and *Streptococcus pyogenes* using a multiplex rapid-cycle PCR with microwell-format probe hybridization. *J Antimicrob Chemother* 2001;**48**:541—4.
16. Jones RN. The emergent needs for basic research, education and surveillance of antimicrobial resistance. Problems facing the report from the American Society for Microbiology Task Force on Antimicrobial Resistance. *Diagn Microbiol Infect Dis* 1996;**25**:153—61.
17. Yoshida R, Kaku M, Kohno S, et al. Trends in antimicrobial resistance of *Streptococcus pneumoniae* in Japan. *Antimicrob Agents Chemother* 1995;**39**:1196—8.
18. Klugman KP. Pneumococcal resistance to antibiotics. *Clin Microb Rev* 1990;**3**:171—96.
19. Linares J, Alonso T, Perez JL, et al. Decreased susceptibility of penicillin-resistant pneumococci to twenty-four β-lactam antibiotics. *J Antimicrob Chemother* 1992;**30**:279—88.
20. Trieu-Cuot P, Poyart-Salmeron C, Carlier C, Courvalin P. Nucleotide sequence of the erythromycin resistance gene of the conjugative transposon Tn1545. *Nuc Acids Res* 1990;**18**:3660—6.
21. Sutcliffe J, Tait-Kamradt A, Wondrack L. *Streptococcus pneumoniae* and *Streptococcus pyogenes* resistant to macrolides but sensitive to clindamycin: a common resistance pattern mediated by an efflux system. *Antimicrob Agents Chemother* 1998;**40**:1817—24.

22. Bryskier A. Novelties in the field of anti-infectives in 1997. *Clin Infect Dis* 1998;**2**:865—83.
23. Morosini M-II., Canton R, Loza E, et al. In vitro activity of telithromycin against Spanish *Streptococcus pneumoniae* isolates with characterized macrolide resistance mechanisms. *Antimicrob Agents Chemother* 2001;**45**:2427—31.
24. Ohkusu K, Nakamura A, Sawada K. Antibiotic resistance among recent clinical isolates of *Haemophilus influenzae* in Japanese children. *Diagn Microbiol Infect* 2000;**36**:249—54.
25. Jacobs MR, Bajaksouzian S, Zilles A, Lin G, Pankuch GA, Appelbaum PC. Susceptibilities of *Streptococcus pneumoniae* and *Haemophilus influenzae* to 10 oral antimicrobial agents based on pharmacodynamic parameters: 1997 US surveillance study. *Antimicrob Agents Chemother* 1999;**43**: 1901—8.
26. Hasegawa K, Yamamoto K, Chiba N, et al. Diversity of ampicillin-resistance genes in *Haemophilus influenzae* in Japan and the United States. *Microb Drug Resist* 2003;**9**:39—46.
27. Catlin BW. *Branhamella catarrhalis*: an organism gaining respect as a pathogen. *Clin Microbiol Rev* 1990;**3**:293—320.
28. Jorgensen JH, Doern GV, Maher LA, Howell AW, Redding JS. Antimicrobial resistance among respiratory isolates of *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae* in the United States. *Antimicrob Agents Chemother* 1990;**34**:2075—80.
29. Doern GV, Bruggemann AB, Pierce G, Hogan T, Holley HP, Rauch A. Prevalence of antimicrobial resistance among 723 outpatient clinical isolates of *Moraxella catarrhalis* in the United States in 1994 and 1995: results of a 30-center national surveillance study. *Antimicrob Agents Chemother* 1996;**40**:2884—6.